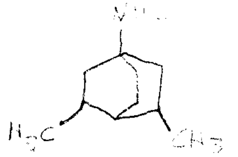
0022-3565/97/2802-0854$03.00/0
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
Copyright © 1997 by The American Society for Pharmacology and Experimental Therapeutics
JPET 280:854–865, 1997



Vol. 280, N
Printed in U.S

Inhibition of Reinforcing Effects of Morphine and Motivational Aspects of Naloxone-Precipitated Opioid Withdrawal by N-Methyl-D-aspartate Receptor Antagonist, Memantine[1]

PIOTR POPIK and WOJCIECH DANYSZ

Institute of Pharmacology, Polish Academy of Sciences, Smętna 12, 31–343 Kraków, Poland (P.P.), and the Department of Pharmacology, Merz+Co. GmbH & CO, Eckenheimer Landstrasse 100–104, D-60318 Frankfurt am Main, Germany (W.D.)

Accepted for publication October 28, 1996

ABSTRACT

The present study focused on the effects of 1-amino-3,5-dimethyladamantane (memantine), a clinically used, low-affinity N-methyl-D-aspartate channel blocker, on the motivational impact of morphine and morphine withdrawal syndrome. Memantine (7.5 mg/kg) inhibited the acquisition as well as the expression of morphine-induced conditioned place preference. However, memantine did not affect significantly the acquisition or expression of conditioned place preference induced by food presentation. In addition, at the dose that blocked morphine-induced conditioned place preference, memantine by itself produced neither conditioned place preference nor conditioned place aversion. Memantine attenuated the negative motivational aspects of morphine withdrawal as assessed by conditioned place aversion produced by a low dose (0.1 mg/kg) of naloxone in morphine-dependent rats. Drug discrimination studies revealed that the inhibitory effects of memantine on morphine-induced conditioned place preference could not be attributed to the attenuation by memantine of the interoceptive cue produced by morphine. In addition, the inhibitory effects of memantine on the expression of morphine-induced conditioned place preference seemed not to be related to effects on memory retrieval, as revealed in the Morris water maze spatial task. These data suggest that memantine at a low, pharmacologically relevant dose of 7.5 mg/kg blocks the reinforcing effects of morphine and aversive effects of morphine withdrawal in rats, which suggests a new potential clinical indication for this agent in the treatment of opioid abuse.

The treatment of drug abuse focuses on prevention of the development of addiction, elimination of existing addiction and suppression of symptoms associated with drug withdrawal (Jaffe, 1987). Current pharmacotherapies usually target specific neurotransmitter systems, which are presumed to mediate the effects of a given class of abused substances. These "targeted," specific pharmacotherapies are used in spite of striking similarities concerning the long-term consequences produced by all of known drugs of abuse such as drug addiction. Moreover, opioid antagonists and agonists are used in the treatment of opioid abuse despite the fact that some therapies (*e.g.*, naltrexone) are not only ineffective, but, in addition, are consistently refused by addicted individuals. In opioid substitution therapy (*e.g.*, with methadone), Ball and Ross (1991) reported a recidivism rate after discontinuation of treatment of ~80%. Dopaminergic pathways, particularly of the mesolimbic system, have been associated with the motivational (reinforcing) aspects of drug abuse. However, to date, antiaddictive therapies targeting dopaminergic neurotransmission have failed to produce a therapeutic breakthrough (Pulvirenti and Koob, 1994).

The pharmacological terms tolerance, sensitization, dependence and withdrawal characterize the clinical term "drug addiction." Drug tolerance refers to the state in which repeated administration of the same dose of a given drug elicits a diminishing effect or the need for an increasing drug dose to produce the same effect. Sensitization (reversed tolerance) refers to the opposite situation in which repeated administration of the same drug dose elicits an escalating effect. Chronic treatment with many drugs, including opioid agonists, produces gradually developing dependence that is defined as the need for continued reexposure to avoid a withdrawal syndrome. The withdrawal syndrome is characterized by physical and motivational disturbances that are the expression of ongoing drug dependence. The term "the maintenance of opioid dependence" refers to the situation in which one can precipitate withdrawal syndrome a long time after

ABBREVIATIONS: CGP-37849, DL-(E)-2-amino-4-methyl-5-phosphono-3-pentanoic acid; CPA, conditioned place aversion; CPP, conditioned place preference; MK-801, (+)-5-methyl-10,11-dihydro-5H-dibenzocyclohepten-5,10-imine maleate, dizocilpine; NMDA, N-methyl-D-aspartate; ANOVA, analysis of variance.

Received for publication July 15, 1996.

[1] This study was partially supported by State Committee for Scientific Research grant 4. PO5A.116.10 to P.P.

the administration of opioid agonist has been discontinued. Contemporary theories of drug addiction consider addictive substances as potent reinforcers. In turn, chronic exposure to these reinforcing drugs can lead to drug addiction, which is best defined as compulsive drug seeking and taking behavior despite adverse consequences. Because such effects are common for all of drugs of abuse (despite the diversity of their chemical structures and plethora of acute pharmacological actions), it is believed that the common neurobiological substrates mediate the motivational impact of drugs producing drug addiction (Eddy *et al.*, 1965; Goudie, 1991; Robinson and Berridge, 1993).

The NMDA receptor complex is a ligand-gated cationic channel, which consists of a pentameric assembly of subunits that contains several regulatory sites. Thus, apart from a recognition site for a primary transmitter (glutamate) it includes sites sensitive to glycine (which is obligatory for receptor activation), polyamines, zinc, redox state and protons (Danysz *et al.*, 1995a; Kutsuwada *et al.*, 1992). It should be noted that agents acting at these sites have not yet been deeply investigated in clinical settings. NMDA receptor function can also be inhibited by blockade of the open ion channel (Danysz *et al.*, 1995a; Kutsuwada *et al.*, 1992). In contrast to the other NMDA antagonists, several open channel (uncompetitive) blockers have been tested in clinical settings, and some of them are being used for medical purposes. Unfortunately, the majority of the high-affinity open channel NMDA receptor antagonists, *e.g.*, phencyclidine or MK-801 (dizocilpine), produce side effects, including confusion, psychotomimetic activity and memory disturbances (Luby *et al.*, 1959; Troupin *et al.*, 1986), that render them unacceptable for a clinical use. In contrast, low-affinity, strongly voltage-dependent uncompetitive NMDA receptor antagonists have been suggested to produce less side effects, perhaps because of weaker effects when the level of NMDA receptor activation is in the physiological range (Chen *et al.*, 1992; Parsons *et al.*, 1993; Rogawski, 1993). One such low-affinity NMDA channel blocker, memantine, has been used clinically for many years in Europe, and is apparently devoid of these side effects if dosed properly, *i.e.*, in gradually increasing doses until therapeutic concentrations are achieved (Ditzler, 1991; Görtelmeyer and Erbler, 1992). A similar favorable therapeutic profile holds true for other agents of this kind, such as amantadine and dextromethorphan (Danysz *et al.*, 1995a; Rogawski, 1993). It seems that the use of NMDA antagonists possessing a relatively credible clinical profile (memantine, dextromethorphan) in novel applications (in this case, the treatment of drug abuse) should be preferred over the use of novel agents that have no such long clinical history.

Converging lines of evidence indicate the essential involvement of NMDA receptors in phenomena related to drug addiction. In preclinical studies, NMDA receptor antagonists decrease tolerance to the locomotor effects of alcohol (Khanna *et al.*, 1993) and sedatives (File and Fernandes, 1994), attenuate sensitization (reverse tolerance) to stimulants (Pudiak and Bozarth, 1993; Wolf and Khansa, 1991) and modify adaptive changes caused by nicotine treatment (Shoaib and Stolerman, 1992). Moreover, NMDA receptor antagonists affect opioid tolerance and dependence processes. Several studies indicated that NMDA receptor antagonists decrease tolerance to the analgesic effects of opiates (Ben-Eliyahu *et al.*, 1992; Bhargava and Matwyshyn, 1993; Elliott *et al.*, 1994; Kolesnikov *et al.*, 1993, 1994; Tiseo and Inturrisi, 1993; Tiseo *et al.*, 1994; Trujillo and Akil, 1991). Similarly, reduction of the physical as well as motivational aspects of the expression of morphine dependence (measured by naloxone-precipitated morphine withdrawal syndrome) has been shown by many investigators (Cappendijk *et al.*, 1993; Higgins *et al.*, 1992; Popik *et al.*, 1995; Rasmussen *et al.*, 1991; Tanganelli *et al.*, 1991; Trujillo and Akil, 1991). In addition, NMDA receptor antagonists inhibit the development of morphine dependence (Elliott *et al.*, 1994; Tiseo and Inturrisi, 1993; Tiseo *et al.*, 1994; Trujillo and Akil, 1991) as well as its maintenance (Popik and Skolnick, 1996). "Antiaddictive" effects of NMDA receptor antagonists (dextromethorphan, ibogaine) have been reported also in initial clinical trials (Koyuncuoglu and Saydam, 1990; Lotsof, 1995).

In spite of intensive efforts, the mechanism of the inhibitory effects of NMDA receptor antagonists in various measures modeling drug addiction remains highly speculative. This is in part caused by the fact that the molecular and physiological mechanism(s) underlying drug dependence and addiction are *per se* not well understood. Although drug dependence (defined by withdrawal syndrome) is traditionally considered to be a major factor in the maintenance of compulsive drug use (Eddy *et al.*, 1965), contemporary theories stress the importance of the reinforcing (incentive) properties of abused substances (Robinson and Berridge, 1993) in the development and maintenance of drug addiction. Thus, it might be hypothesized that antiaddictive treatments should inhibit ongoing drug-seeking through a decrease of the reinforcing impact of drugs of abuse, rather than by attenuating solely the severity of the withdrawal syndrome.

Recently, Bespalov *et al.* (1994) demonstrated that the nonselective glutamate receptor antagonist, kynurenic acid, attenuates the acquisition and expression of CPP induced by morphine. The same treatment inhibits morphine-induced facilitation of responding in the electrical intracranial self-stimulation paradigm (Bespalov *et al.*, 1994). Similar attenuation of the development of morphine-induced CPP has been recently shown for MK-801 (dizocilpine) and CGP-37849, which are uncompetitive and competitive NMDA receptor antagonists, respectively (Tzschentke and Schmidt, 1995). The ability to induce CPP and to facilitate intracranial self-stimulation, as well as to induce and maintain self-administration, defines in animal models reinforcing properties of drugs and, according to several theories of drug addiction, potential abuse in humans (for review, see Goudie, 1991). Thus, it seems likely that treatments decreasing these reinforcing actions of drugs in animal models would be effective in diminishing intake of drugs of abuse in humans.

CPP offers a reliable measure for assessing the reinforcing value of pharmacological treatments and other reinforcers, including food. In the case of pharmacological manipulations, during training a drug supposed to have reinforcing properties is paired with a distinctive compartment, whereas its vehicle is paired with the other compartment of the same training apparatus. After completing the association phase, drug-free animals explore both compartments. It is assumed that an increase of the time spent in a compartment associated with a given treatment reflects the incentive (positively reinforcing) value of that treatment (Bindra, 1978), whereas a decrease suggests aversive properties (Carr *et al.*, 1989;

Mucha *et al.*, 1982). Numerous studies indicate that in addition to the reinforcement, associative learning must occur for the development of CPP (*e.g.*, White and Carr, 1985). CPP induced by opiates is stereospecific, can be blocked by opioid antagonists and is probably mediated by action at *mu* rather than *kappa* opioid receptors (Mucha and Herz, 1985). In addition, dopaminergic mesolimbic pathways have been reported to participate in opioid-induced CPP (Spyraki *et al.*, 1983).

The objective of the present experiments was to determine whether the NMDA receptor antagonist memantine could affect the reinforcing impact of morphine as well as the motivational aspects of morphine withdrawal in a CPP paradigm. Memantine was selected for the present studies, because it is a use-dependent NMDA receptor antagonist (IC_{50}= 0.5–3.0 μM [Bormann, 1989; Chen *et al.*, 1992; Kornhuber *et al.*, 1989; Parsons *et al.*, 1993] that currently is clinically used in Germany in the treatment of senile dementia and spasticity (Ditzler, 1991; Görtelmeyer and Erbler, 1992). More specifically, memantine's effects on both acquisition and expression of CPP induced by morphine were assessed in the first experiment. The specificity of memantine's effect was tested subsequently in the CPP paradigm in which rats were reinforced by food, rather than morphine injection. The next experiment was carried out to find if memantine may influence the motivational aspect of naloxone-precipitated morphine withdrawal, as measured in the CPA paradigm. Because the impact of memantine on acquisition/expression of CPP could also be explained by its effects on learning and memory, experiments were performed in which memantine was given to rats acquiring and retrieving spatial information in the water maze. In addition, because the effects of memantine on morphine-induced CPP could be interpreted as a diminution of the subjective (interoceptive) effect of morphine, in the last experiment we investigated the influence of memantine on the interoceptive cue produced by morphine in a drug-discrimination paradigm.

Materials and Methods

Subjects. Male Wistar rats (approximately 300 g of weight at the beginning of the experiment) were housed under standard laboratory conditions for at least 2 weeks before experiments started. Animals were kept in plastic cages, four rats per cage (58 × 37 × 19 cm) in the animal room with a controlled light-dark cycle (lights on, 7:00 A.M.; off, 7:00 P.M.). Water and commercial food were available *ad libitum*, unless otherwise stated.

Drugs. Morphine HCl (Polfa), naloxone HCl (Endo), memantine HCl (Merz and Co.), (+)-morphine and etonitazene (both gifts of Dr. K. Rice, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD) were dissolved in physiological saline. Saline was used as placebo. The doses of morphine, naloxone and etonitazene correspond to the doses calculated as base for all other agents as respective salts. All injections were given in a volume of 1 ml/kg i.p., except in the drug discrimination experiment in which all agents except memantine were injected subcutaneously.

Induction of CPP. The CPP procedure was similar to that described previously by Papp and Moryl (1994). Four identical wooden boxes with white and black chambers (30 × 20 × 25 cm each) were used. The chambers had distinct floor textures (plain wood in the white chamber and wire mesh in the black chamber, respectively). The gray central area (12 × 20 × 25 cm) constituted a "neutral" chamber. The CPP procedure consisted of *adaptation*, *pretest*, acquisition of a conditioned response and the *posttest*. During the first 3 days of training (adaptation phase), the rats were placed individually in the apparatus to freely explore it for 10 min daily. These trials as well as preliminary studies indicated that almost all subjects preferred the black chamber over the white one. On day 4 (pretest), the time spent in the white chamber during a 10-min free exploration session was measured and recorded. This measure was used as an initial preference score for each subject.

To measure the effects of memantine on the *acquisition* of morphine-induced CPP, on days 5, 7 and 9, rats were injected with placebo and then with vehicle, 3.75 or 7.5 mg/kg of memantine, 15 and 30 min, respectively, before being placed in the black chamber of the apparatus for a trial lasting 30 min. On days 6, 8, and 10, rats were injected with corresponding doses of memantine followed 15 min later by morphine administration (1 mg/kg); 15 min later, rats were placed in the white chamber for a trial lasting 30 min. These 6 consecutive days served as conditioning trials. Changes in the CPP scores were measured on day 11 (posttest) when rats were injected with placebo 20 min before being placed in the apparatus. Again, the time spent in the white chamber was recorded during the session lasting 10 min.

A similar procedure was used to measure the effects of memantine on the *expression* of morphine-induced CPP, with the exception that rats were injected with morphine during acquisition trials and by vehicle, 3.75 or 7.5 mg/kg of memantine, 20 min before the posttest.

In a variation of this procedure, another group of rats was reinforced with food pellets instead of morphine injections. Three weeks before the experiment began, these subjects were maintained with restricted food access and were fasted for 12 to 16 hr before the test. As described above, the experiment began with a adaptation phase and pretest. On days 5, 7 and 9, rats were injected with vehicle, 3.75 or 7.5 mg/kg of memantine, 30 min before being placed in the black chamber of the apparatus for a trial lasting 30 min. On days 6, 8 and 10, rats were injected with corresponding doses of memantine 30 min before being placed in the white chamber. In the white chamber, each rat was offered five standard laboratory food pellets (~11 g). The amount eaten by each subject during each of the food-white chamber pairings was measured. Starting 30 min after the conditioning sessions, rats were returned to their home cages, and they were allowed to eat standard laboratory food until 12 to 16 hr before the next trial began. In the same experiment an additional group was used to evaluate the effects of memantine in the expression of food-induced CPP. The procedure was the same as described above with the exception that memantine was not given before every conditioning trial but 20 min before the posttest (at the dose of 3.75 or 7.5 mg/kg). There were 10 to 15 rats in each group undergoing the CPP procedure.

Induction of CPA. The CPA procedure was conceptually based on the work of Higgins and colleagues (1991, 1992). Rats were injected with morphine (10 mg/kg, twice daily, at 9:00 A.M. and 5:00 P.M.) for 8 days. On days 3, 4 and 5 of morphine treatment, adaptation sessions (10 min of unobserved, free exploration) were carried out at least 2 hr after the morning dose of morphine. On day 6 of morphine treatment, the pretest session was performed, as described for the CPP procedure, with the exception that the time in the black chamber was measured. On day 7, at least 90 min after the morning injection of morphine, rats were injected with placebo and 30 min later again injected with placebo. Immediately after the second injection, subjects were placed in the white chamber of the apparatus for 30 min. On day 8, at least 90 min after the morning injection of morphine, rats were injected with vehicle, 3.75 or 7.5 mg/kg of memantine, and 30 min later they received a 0.1 mg/kg naloxone injection. Immediately after the naloxone injection, rats were placed in the black chamber of the apparatus for 30 min. No more morphine injections were given to the animals. On day 9, the posttest was performed, during which the time spent in the black chamber was recorded during a session lasting for 10 min. The number of rats in each group was 10 to 15.

Effects of memantine on the acquisition and retention of spatial learning and memory. Rats were trained to find a metal platform that was submerged 1 cm below the water surface in the swimming pool (50 cm high, 180 cm in diameter) (Popik *et al.*, 1994a,b). The platform was positioned half-way between the wall and the center of the circular pool and remained in this position throughout the 4 training days. There were six trials on each training day. Each trial started from one of the four compass points around the pool perimeter, with the sequence, *e.g.*, N, E, S, W, N, E. Rats were gently placed into the water, facing the wall; the latency to find the platform was measured for each rat. Subjects were kept on the platform for 30 sec, after which the next trial started. After completion of the six swimming trials, rats were transferred to the "drying" cage and later to their home cages. The experimental room contained numerous visual cues and had dispersed illumination that allowed videotaping.

Rats were treated with either placebo (physiological saline, n = 16) or memantine (7.5 mg/kg, n = 8), each administered daily, 30 min before the first trial. After 4 training days, placebo-treated rats were divided in two groups that matched the total number of seconds across the whole 24 trials (a raw measure of performance); and, therefore, the statistics and presentation of data include one learning curve of memantine-treated and two curves of placebo-treated subjects. On the fifth day of training, a probe trial was carried out. The probe trial consisted of 1 min of videotaped observation of swimming behavior in the tank with the platform removed. The analysis of swimming paths was carried out off-line with use of EYE (J. Dlugopolski, Kraków, Poland) and TRACK-ANALYZER (Wolfer and Lipp, 1992) software. Half of placebo-treated rats (n = 8) were treated 20 min before being placed into the pool with placebo, and the remaining rats (n = 8) received memantine (7.5 mg/kg) injection. Rats that were administered memantine during learning trials received placebo injection before the probe trial.

Effects of memantine on morphine-produced interoceptive cue as measured in a drug-discrimination paradigm. The Morris water maze as described above was used to train rats (n = 8) to discriminate between placebo (physiological saline) and (−)-morphine HCl interoceptive cue (3.5 mg/kg). Rats were injected with placebo or morphine 20 to 30 min before being gently placed in the tank at either NE or SW compass starting point. Subjects were given four trials per day, usually 6 to 7 days a week. The sequence of starting points was random throughout the experiment and independent from treatment conditions; however, in a given day a sequence of either the NE, SW, NE, SW or SW, NE, SW, NE of starting points was used. During the training, only one submerged platform was present inside the tank and only one treatment was given. Half of the subjects were required to associate placebo injection with the platform positioned in the NW quadrant and morphine injection with the platform positioned in the SE quadrant. For the remaining half of the subjects, the assignment of platforms was reversed. Typically, a double alternation scheme of training was used, with rats receiving placebo-placebo-morphine-morphine injections on subsequent days. Subjects were kept on the platform for 30 sec, after which the next trial started. After completion of the four swimming trials, rats were transferred to the "drying" cage and later to their home cages. It took approximately 40 training days for a rat to achieve the criterion of "good" performance, defined as eight out of nine consecutive *first* correct swimming trials. A trial was considered "correct" if the rat swum from the starting point to the respective platform without swimming in the vicinity (an area about two times bigger than the size of platform) of the platform associated with the alternative treatment (see fig. 1 for details).

The accuracy to find the platform (*i.e.*, the presence or absence of an error defined by swimming to the incorrect platform position) and latency to find the platform was measured for each rat during every trial.

In contrast to the "training" days, during the "testing" days, two identical submerged platforms were placed in the pool in the positions that had been previously associated with placebo and morphine training dose (3.5 mg/kg) injections. Only one swimming trial was given on the "testing" day. Rats were kept on the platform for 30 sec, after which they were transferred to the "drying" cage.

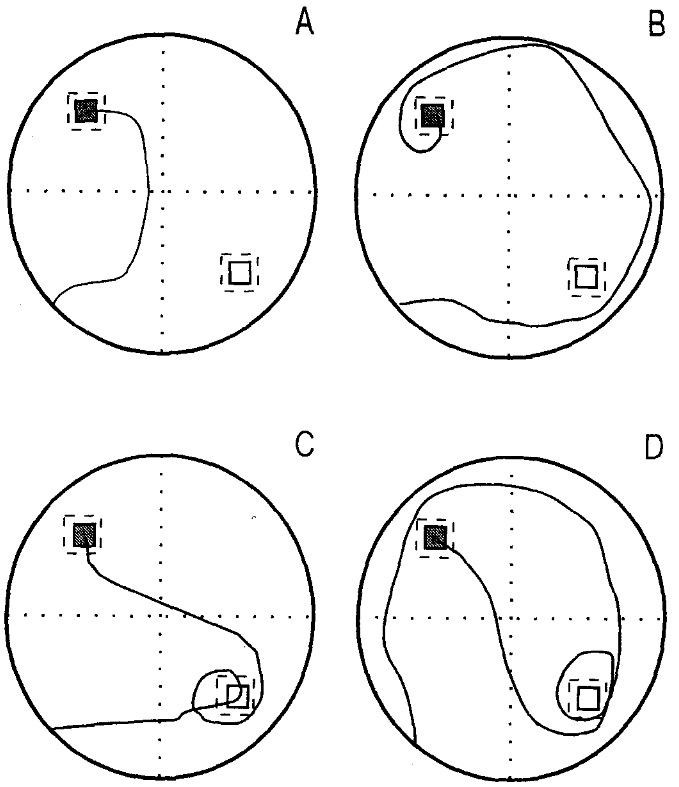


Fig. 1. Images of the typical swimming paths of rats trained to discriminate morphine from placebo in the Morris water maze. Presented are layouts of the experimental setup. Smaller rectangles represent actual platform positions, with shaded smaller rectangles representing "correct" and open smaller rectangles representing "incorrect" platform positions, respectively. The bigger dashed rectangles around "incorrect" platform represent area that, if encountered by a subject, indicated erroneous trial. A and B images represent error-free trials, and C and D images represent erroneous trials.

After a rat was considered well trained, first, a dose-response curve with morphine (3.5, 2.625, 1.75 and 0.875 mg/kg) was constructed both the day after placebo and the day after the training dose (3.5 mg/kg) of morphine. Higher than 3.5 mg/kg doses of morphine produced impairment of swimming behavior and therefore were not used. After construction of the dose-response curves: 1) the generalization to an etonitazene cue, 2) the stereospecificity of the morphine cue and 3) the blockade of the morphine (1.75 mg/kg) cue by naloxone (0.05 mg/kg) were assessed to check out the specificity of the method. Finally, the generalization by memantine (3.75 and 7.5 mg/kg) to the morphine-produced interoceptive cue as well as the effects of memantine (3.75 and 7.5 mg/kg) pretreatment on the morphine (1.75 mg/kg) interoceptive cue were measured.

Data analysis and statistics. In the CPP and CPA studies, the preference scores were expressed as a percent increase or decrease of time spent by a rat in a given chamber of the apparatus on pretest and posttest. Data were analyzed by ANOVA, followed by Duncan or Student-Newmann-Keuls tests.

The changes in the latencies to find the platform during learning trials in the water maze task were analyzed by repeated measures ANOVA. One-way between subjects ANOVAs were used to analyze several parameters of the paths recorded during the probe trial.

For establishing the ED_{50} dose of morphine in the drug-discrimination paradigm, the Litchfield and Wilcoxon (1949) procedure was used. The frequency data from the discrimination study were analyzed by the Fisher's Exact Probability Test.

Results

Effects of memantine on the CPP induced by morphine. During the CPP pretest there were no differences in the time spent in the white chamber of the apparatus among groups (ANOVA, P = .23). However, the preference scores to that chamber after conditioning varied greatly among groups (*F*(8,97) = 4.09, P = .0003). Rats injected with morphine in the white (initially nonpreferred) chamber demonstrated a marked preference for this chamber during the drug-free posttest. To assess the effects of memantine on the acquisition of CPP, rats were injected with memantine before every conditioning session.

Memantine dose-dependently attenuated the acquisition of morphine-induced CPP, because at a dose of 7.5 mg/kg it produced statistically significant diminution of CPP as compared with placebo treatment (fig. 2). To assess the effects of memantine on the expression of CPP, rats that were previously conditioned to morphine received memantine (1.88, 3.75 or 7.5 mg/kg) before the posttest. Memantine dose-dependently attenuated the expression of morphine-induced CPP (fig. 2). As in the acquisition study, a dose of 7.5 mg/kg produced statistically significant inhibition of CPP expression. Control experiments demonstrated that rats injected in the white chamber with memantine (7.5 mg/kg) instead of morphine did not acquire *preference* to the memantine-associated chamber (fig. 2, dotted bar). In addition, memantine given at the same dose did not produce *aversion* to the memantine-associated chamber. Thus, whereas although rats treated with placebo in the black chamber demonstrated 76.8 ± 8.3% (*n* = 12) of the preference to that compartment, subjects treated with memantine in the black chamber demonstrated 76.6 ± 24.7% (*n* = 10) preference on the posttest compared with their pretest values.

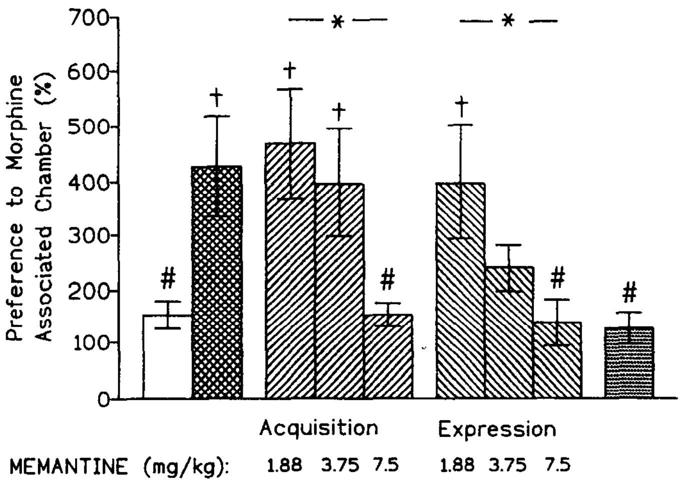


Fig. 2. The effects of memantine on acquisition and expression of morphine-induced CPP. Presented are preference scores for the white chamber of the apparatus after 6 days of conditioning. Open bar indicates scores of placebo-conditioned control subjects (*i.e.*, rats that received 6 placebo injections during conditioning and placebo injection before the posttest). The double hatched bar represents scores of morphine-conditioned control subjects. The hatched bars represent scores of rats that received memantine, at doses indicated, before every conditioning trial and placebo before the posttest (left panel), or placebo before every conditioning trial and memantine before the posttest (right panel). The dotted bar represents scores of rats conditioned with 7.5 mg/kg of memantine instead of morphine. The number in each group is 10 to 15 rats. Values are mean ± S.E.M. Symbols: #P < .05 *vs.* morphine-conditioned subjects; †P < .05 *vs.* placebo-conditioned controls; *P < .05 *vs.* the respective group, Student-Newman-Keuls test.

Effects of memantine on the CPP induced by food. Rats that received placebo injections both during conditioning and before the posttest acquired CPP to the chamber associated with food (fig. 3).

Memantine at the doses tested (3.75 and 7.5 mg/kg) did not influence significantly the acquisition or expression of food-induced CPP (*F*(4,47) = 1.480; P = .2249). Interestingly, memantine dose-dependently inhibited consumption of food during the conditioning sessions (fig. 4).

Two-way ANOVA demonstrated significant effects of the dose (*F*(2,90) = 16.25; P < .001) and the day (*F*(2,90) = 5.56; P = .0053), but no interaction. *Post hoc* comparison by Student-Newman-Keuls test revealed that memantine at 3.75 as well as at 7.5 mg/kg produced a statistically significant decrease in the amount of food consumed compared with placebo. Further analysis revealed that this decreasing effect on food consumption was tolerated during the time of the experiment.

Effects of memantine on the CPA induced by naloxone-precipitated morphine withdrawal. Placebo-pretreated, naloxone-challenged control rats demonstrated ~75% of their initial preference to the black chamber, whereas morphine-dependent naloxone-challenged animals showed significantly lower preference to that chamber (fig. 5). ANOVA demonstrated significant differences among groups (*F*(4,51) = 2.91; P = .031).

These data indicate the occurrence of CPA to the morphine-withdrawal-associated chamber. Memantine tended to attenuate this aversion, although the preference score calculated for rats treated even with the highest dose of meman-

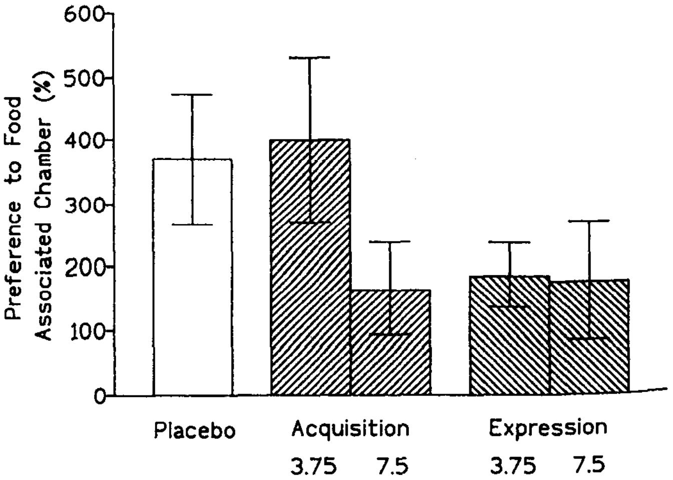


Fig. 3. The effects of memantine on acquisition and expression of food-induced CPP. Presented are preference scores for the white chamber of the apparatus after 6 days of conditioning. Open bar indicates scores of placebo-conditioned control subjects (*i.e.*, rats that received 6 placebo injections during conditioning and placebo injection before the posttest). Hatched bars represent scores of rats that received memantine, at doses indicated, before every conditioning trial and placebo before the posttest (left panel), or placebo before every conditioning trial and memantine before the posttest (right panel). The number in each group is 10 to 15 rats. Values are mean ± S.E.M. ANOVA indicated no effects of memantine treatment.

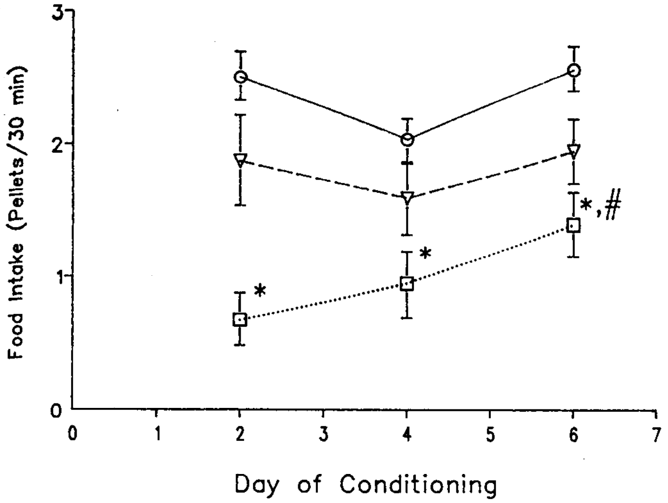


Fig. 4. The effects of memantine on food intake during acquisition of food-induced CPP. Presented are mean ± S.E.M. number of pellets consumed by rats during conditioning days 2, 4 and 6. On these tests, rats were offered five food pellets in the white chamber of the apparatus. Rats received placebo (circles) or memantine at doses 3.75 (triangles) or 7.5 (squares) mg/kg, respectively, 30 min before being inserted into the apparatus. Symbols: *P < .05 *vs.* placebo-treated (Student-Newmann-Keuls test, after one-way ANOVA); #P < .05 *vs.* memantine 7.5 mg/kg treated on day 2 (paired *t* test).

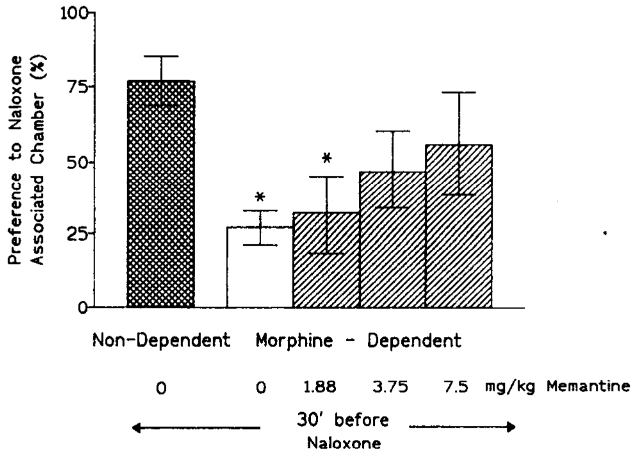


Fig. 5. The effects of memantine on acquisition of naloxone-precipitated CPA in morphine-dependent rats. Presented are preference scores for the black chambers of the apparatus after 2 days of conditioning. The double-hatched bar indicates the scores of control rats that were not morphine-dependent and received placebo injections during conditioning. The open bar represents the scores of morphine-dependent control subjects. The hatched bars represent scores of morphine-dependent rats that received memantine at the doses indicated on the bottom of the figure. The number in each group is 10 to 15 rats. Values are mean ± S.E.M. Symbols: *P < .05 *vs.* control, morphine-nondependent, placebo-treated rats, Student-Newman-Keuls test.

tine (7.5 mg/kg) was not significantly different from that of rats treated with placebo. However, only placebo- and memantine (1.88 mg/kg)-treated, morphine-dependent rats demonstrated significant CPA, which indicated partial inhibition by higher doses of memantine.

In the CPA test, rats did not express overt physical symptoms of morphine withdrawal (wet dog shaking, jumping, etc.), perhaps because of the fact that the dose of naloxone used was relatively low (0.1 mg/kg) compared with that required to elicit overt withdrawal syndrome (*e.g.*, Brent and Chahl, 1993; Cappendijk *et al.*, 1993). However, because of the experimental conditions, these behaviors were not measured explicitly.

Morris water maze procedure: Effects of memantine on acquisition and retention of spatial learning and memory. All placebo-treated rats trained in the Morris water maze demonstrated rapid acquisition of the spatial memory (fig. 6). Two-way repeated measures ANOVA performed on the swimming latencies data for all 24 trials demonstrated significant effects of the treatment ($F(2,483) = 16.54$; $P < .001$), trial number ($F(23,483) = 26.88$; $P < .001$) and interaction ($F(46,483) = 1.92$; $P < .001$). Significant differences among groups (memantine *vs.* vehicle) were detected when data were analyzed separately for the first ($P < .001$), second ($P < .001$) and third ($P < .01$), but not the fourth day of training.

The results of the computer-assisted analysis of swimming paths recorded during the probe trial revealed that except for the longer swimming paths of rats treated with memantine during training, no other parameters (in particular, the time spent in the training quadrant) were different among various groups (table 1).

Effects of memantine on morphine-produced interoceptive cue as measured in the drug-discrimination paradigm. Rats tested the day after the morphine training dose responded to the graded doses of morphine with an ED_{50} of 0.74 mg/kg, whereas on the day after placebo training the ED_{50} was 1.23 mg/kg (see table 2 for raw data). These dose-response curves were not different from each other as calculated with the Litchfield and Wilcoxon (1949) procedure. Therefore, the ED_{50} of the combined data from both groups was 0.98 mg/kg with confidence limits of 0.64 to 1.51 mg/kg.

Only 1 of 8 rats selected the "morphine-positive" platform when injected with 0.05 mg/kg naloxone 5 min after the test dose of morphine (1.75 mg/kg) (table 2). The number of rats responding positively to 1.75 mg/kg morphine injection as compared with the number of rats positively responding to

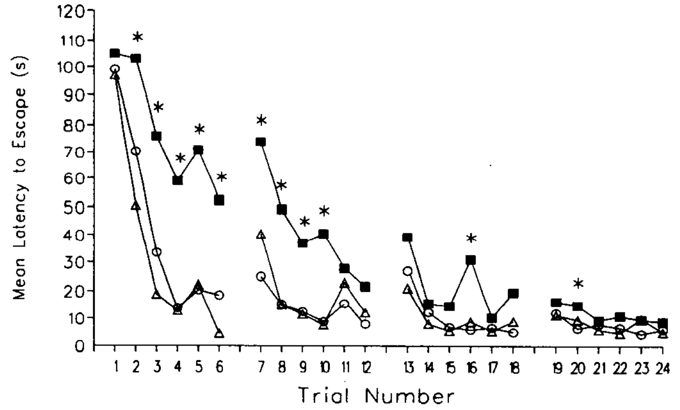


Fig. 6. Effects of memantine on the acquisition and retrieval of the spatial learning task. Presented are mean escape latencies of rats pretreated with 7.5 mg/kg of memantine (squares) or placebo (circles or triangles) 30 min before each of the first learning trials (*i.e.*, trials 1, 7, 13 and 19). Asterisks indicate that on a given trial rats treated with memantine demonstrated significantly longer latencies to find the platform than placebo-treated subjects on the given trial (P < .05, Student-Newman-Keuls test, after one-way ANOVA). No differences were detected between the placebo-treated groups. S.E.M. is not shown for the clarity of the figure.

TABLE 1

The analysis of swimming paths of rats trained for 4 days in the Morris water maze and performing the probe trial (mean ± S.E.M.)

Rats ($n = 8$ in each group) were treated with [placebo + placebo], [placebo + memantine] or [memantine + placebo] before each of the four learning trials and before the probe trial, respectively. Memantine was used at the dose of 7.5 mg/kg.

Parameter	Pretreatment before learning-Treatment before probe trial			ANOVA $F(2,21)$
	Placebo-Placebo	Placebo-Memantine	Memantine-Placebo	
Time in the training quadrant (sec)	21.2 ± 2.8	20.2 ± 2.0	23.2 ± 2.4	<1
Platform position hits	3.1 ± 0.5	2.0 ± 0.8	1.6 ± 0.4	1.724
Path (cm)	1442 ± 53	1434 ± 54	1641 ± 63*	4.24, $P < .05$
Activity (sec)	59.0 ± 0.2	58.4 ± 0.6	59.5 ± 0.2	2.07
Mean distance to the wall (cm)	28.3 ± 2.1	31.1 ± 3.0	25.1 ± 1.9	1.59

* $P < .05$ *vs.* all other groups (Student-Newmann-Keuls test).

TABLE 2

Morphine-produced drug discrimination in rats trained to discriminate (−)-morphine from placebo in the Morris water maze

Morphine Dose	Number of Rats Positively Choosing Morphine-Associated Platform/Tested			
	Day after morphine training dose	Day after placebo	Day after morphine and placebo	+ Naloxone (0.05 mg/kg)
mg/kg				
0	0/8	0/8	0/16	
0.875	5/8	3/8	8/16	
1.75	6/8	5/8	11/16	1/8*
2.625	7/8	6/8	13/16	
3.5	8/8	8/8	16/16	
ED$_{50}$[a]	0.74 (0.38–1.43)	1.24 (0.79–1.91)	0.98 (0.64–1.51)	

[a] ED$_{50}$ values are stated as milligrams per kilogram with confidence limits in parentheses.

* $P < .05$ *vs.* all groups treated with 1.75 mg/kg of morphine (columns 2, 3 and 4) (Fisher's Exact Probability Test).

combination of morphine (1.75 mg/kg) and naloxone (0.05 mg/kg) injections was statistically different (Fisher's Exact Probability Test), which indicates that naloxone blocked morphine interoceptive cue in this paradigm.

The reliability of the present discrimination paradigm was assessed by an additional experiment assessing the effect of etonitazene. Etonitazene produced a dose-related increase in choosing the morphine-positive platform with an ED$_{50}$ of 0.00189 mg/kg (confidence limits, 0.00129–0.00277), being 3 orders of magnitude more potent than (−)-morphine (table 3). In contrast, 3.5 mg/kg of (+)-morphine did not produce morphine-positive responses. Higher doses of (+)-morphine were not used because of shortage of the material.

Further experiments were designed to test the possibility that memantine (3.75 and 7.5 mg/kg) substitutes for the morphine cue and/or modifies the morphine (1.75 mg/kg) interoceptive cue. In this test, 0/8 and 1/8 of rats selected the morphine-positive platform when treated with 3.75 and 7.5 mg/kg of memantine, respectively (table 4). Moreover, the number of rats responding positively to 1.75 mg/kg morphine injection was not statistically different from the number of rats positively responding to memantine (3.75 or 7/5 mg/kg) and morphine (1.75 mg/kg) joint treatment.

Discussion

The present findings demonstrate that memantine in a dose-dependent fashion attenuated the acquisition and expression of morphine-induced CPP, but had no significant effect on the acquisition or expression of CPP induced by food

TABLE 3

Substitution to etonitazene and (+)morphine in rats trained to discriminate (−)-morphine from placebo in the Morris water maze

Drug Dose[a]	Number of Rats Positivity Choosing Morphine-Associated Platform/Tested	
	Etonitazene	(+)Morphine
0	0/8	
0.875	2/8	
1.75	2/8	
2.625	5/8	
3.5	7/8	1/8*
ED$_{50}$[b]	0.00189 (0.00129–0.00277)	

[a] The drug dose for etonitazene is in micrograms per kilogram. The drug dose for (+)morphine is in milligrams per kilogram.

[b] The ED$_{50}$ value is stated in milligrams per kilogram with confidence limits in parentheses.

* $P < .05$ *vs.* groups treated with 3.5 mg/kg of morphine (shown in table 2) (Fisher's Exact Probability Test).

TABLE 4

Effects of memantine on morphine-produced interoceptive cue in the Morris water maze

Memantine	Number of Rats Positively Choosing Morphine-Associated Platform/Tested	
	Vehicle	Morphine (1.75 mg/kg)
mg/kg		
0		11/16
3.75	0/8	7/8
7.5	1/8	6/8

presentation. Moreover, memantine partially inhibited acquisition of motivational aspects of naloxone-precipitated morphine withdrawal as measured in the CPA paradigm. In addition, at the dose that blocked morphine-induced CPP, memantine by itself produced neither CPP nor CPA. We failed to demonstrate that memantine at doses effective in CPP and CPA paradigms modified the interoceptive cue produced by morphine. Although, initially, memantine attenuated the acquisition of spatial learning, this effect was tolerated after 3 days, and no interference with the retrieval of spatial memory was found in the present experiments.

Consonant with previous studies (for reviews see Carr *et al.*, 1989; Goudie, 1991) the present experiments revealed that morphine produced clear-cut CPP. Memantine inhibited both the acquisition and expression of morphine-induced CPP. Similar inhibition was found previously for kynurenic acid (Bespalov *et al.*, 1994), a nonselective antagonist of excitatory amino acid receptors. Recently, Tzschentke and Schmidt (1995) demonstrated that dizocilpine and CGP 37849, uncompetitive and competitive NMDA receptor antagonists, respectively, attenuate the acquisition of morphine-induced CPP.

The reduction of morphine-induced CPP by an NMDA receptor antagonist (memantine) found in the present study could be related to several effects that were not explicitly ruled out in the previous studies. First, uncompetitive NMDA receptor antagonists are known to exert inhibitory effects on associative learning (see Danysz *et al.*, 1995b, for review). Because the establishment of CPP involves associative learning, it is likely that treatments inhibiting plastic processes would inhibit CPP as well. However, it is generally agreed that NMDA receptor antagonists affect the acquisition of new information but not the storage or recall of associations that are well established (Caramanos and Shapiro, 1994; Danysz *et al.*, 1995b; Shapiro and Caramanos, 1990). Moreover, the amnestic effects of memantine are usually seen at higher doses (10–20 mg/kg; Misztal *et al.*, 1995), which suggests that the learning impairment is not of major importance for antagonism of morphine-induced CPP in which a lower dose was used. Although memantine initially increased latencies to find the hidden platform (which likely reflects disturbances in swimming behavior and/or initial attenuation of spatial learning acquisition), these effects were not longer apparent on the last day of training (fig. 6). Thus, even if memantine were initially to impair the acquisition of morphine-induced CPP, the purported amnestic actions of this compound, by analogy, should cease by day 3 of conditioning, whereas the association phase in the CPP experiments lasted for 6 days. Further evidence against strong amnestic effects comes from the analysis of swimming paths recorded during the probe trial that specifically measures the strength of spatial memory. Thus, table 1 demonstrates no differences between spatial navigation behavior of rats treated with memantine or placebo during the acquisition of the Morris spatial task. In agreement with our data, Barnes *et al.* (1996) reported that rats treated chronically with food containing memantine (30 mg/kg/day) show increased maintenance of long-term potentiation *in vivo* and normal learning in the Morris maze. Other studies indicate that memantine infused at doses leading to serum concentrations observed in humans has no effects in naive animals and actually improves radial maze learning in rats after entorhinal cortex lesion (Zajaczkowski *et al.*, 1996). Moreover, Ditzler (1991), as well as Görtelmeyer and Erbler (1992), reported positive cognitive effects of memantine in demented patients.

Memantine at the dose inhibiting acquisition of morphine-induced CPP did not significantly affect food-induced CPP (fig. 3), which suggests that memantine-treated rats were able to associate food reinforcement with a distinctive environment. It should be mentioned that although statistical analysis (ANOVA, $P > .05$) indicated that memantine affected neither acquisition nor expression of food-induced CPP, the data presented in fig. 3 suggest that inhibitory effects of memantine might have been detected if a different statistical approach had been used. Such inhibitory effect may likely be related to the initial decrease of food intake seen in memantine-treated rats (fig. 4) and/or initial attenuation of learning processes as revealed in the Morris water maze (fig. 6). It remains an open question whether these factors contributed to memantine-induced attenuation of acquisition of morphine-induced CPP (fig. 2). Nonetheless, these factors are unlikely to explain the inhibitory effects of memantine on the expression of morphine-induced CPP, because memantine did not affect the retrieval of spatial memory (table 1).

It has been suggested recently that the treatments attenuating the rewarding aspects of drugs of abuse may have aversive properties by themselves, which confounds the interpretation of the results of CPP studies. Such aversive effects were demonstrated for the L-type calcium channel blockers (Pizzi and Cook, 1996) which inhibit, *e.g.*, cocaine-induced CPP (Pani *et al.*, 1991). To rule out this interpretation, the experiment was performed in which memantine (7.5 mg/kg) was administered to the rats in the black compartment of CPP apparatus. If memantine would have aversive actions, it is likely that rats would avoid the memantine-associated chamber. The data presented under "Results" demonstrate the lack of aversive effects of memantine, thus making the "aversive" interpretation unlikely.

Memantine could also decrease morphine-induced CPP by attenuating the interoceptive cue produced by morphine and perhaps related to its reinforcing action. Morphine possesses clear discriminative properties (Colpaert, 1977; Shannon and Holtzman, 1979; Young *et al.*, 1992) that are thought to contribute to its abuse liability (Jaffe, 1987; Martin and Jasinski, 1969). The correct choice of the drug-associated compartment in the CPP procedure is based, among others, on the recognition of *spatial* cues, whereas the most often used procedures for measuring discriminative properties of drugs are based on *operant* conditioning. Spatial learning and operant conditioning tasks use different forms of memory; and, perhaps because of this fact, they are affected differently by amnestic treatments (Danysz *et al.*, 1995b; Wessinger, 1994). It was therefore reasonable to compare the effects of memantine on morphine-induced CPP in a task relying on similar cognitive processes. Thus, for the present experiments a drug-discrimination procedure has been developed that is based on the ability of rats to associate the spatial position of a hidden platform with the interoceptive cue produced by the drug. Spatial learning paradigms based on water T-maze learning have been used previously for studying discriminative effects of drugs (Henriksson and Jarbe, 1972; Jarbe, 1987).

Several earlier studies indicate that the cue produced by morphine in drug discrimination paradigms arises from central drug actions and is independent from its peripheral effects (Colpaert *et al.*, 1975; Gianutsos and Lal, 1975, 1976). In the drug discrimination procedure used in the present study, rats were able to discriminate 3.5 mg/kg of morphine from placebo, a finding in accordance with numerous previous studies that used operant conditioning techniques. To check the reliability of the present version of the drug-discrimination paradigm, we investigated whether the discriminative effect of morphine: 1) is similar 1 day after morphine treatment as well as 1 day after placebo treatment; 2) is stereospecific; 3) if another opioid *mu* agonist will substitute for the morphine interoceptive cue and 4) if the discriminative effect of morphine could be blocked by a specific antagonist. The data presented (tables 2 and 3) demonstrate that the water maze could be used successfully as a tool for studying discriminative effects of drugs. Thus, 1) the ED_{50} value for the morphine interoceptive cue appeared not to be different the day after the morphine training dose from the day after placebo treatment; 2) (+)-morphine, an unnatural isomer that is devoid of opioid effects (Adams *et al.*, 1991; Van der Kooy *et al.*, 1982) did not substitute for the (−)-morphine interoceptive cue; and 3) etonitazene, a *mu* agonist $\sim$1000 times more potent than morphine in behavioral and neurochemical assays (Rice *et al.*, 1983; Wikler *et al.*, 1963) substituted for the morphine interoceptive cue with respectively higher potency than morphine itself. In addition, a low dose of naloxone abolished the discriminative cue produced by morphine. However, subjects injected with morphine and memantine behaved similarly to rats injected with morphine only (table 4). Thus, the present data suggest that it is unlikely that memantine decreased the morphine interoceptive cue and thereby inhibited acquisition of morphine-induced CPP. In agreement with our data, Bespalov and colleagues (1995) reported recently in abstract form that a nonselective glutamate receptor antagonist, kynurenic acid, did not affect the heroin discriminative cue.

Since most probably, memantine did not inhibit the acquisition of morphine-induced CPP through learning impairment or attenuation of the morphine interoceptive cue, it is likely that it attenuated the reinforcing impact of morphine. This observation is supported by the fact that memantine also inhibited the expression of morphine-induced CPP, which suggests that this compound diminished the conditioned (secondarily reinforcing) properties of the morphine-associated environment. The inhibitory action of NMDA receptor antagonists on the reinforcement produced by morphine are far from being fully understood. Nevertheless, the inhibitory effects of memantine on morphine-induced CPP but lack of effect on morphine discrimination may be explained by differential involvement of dopaminergic transmission in these two phenomena. For example, it is generally agreed that opioid (morphine and heroine)-induced CPP depends critically on dopamine mesolimbic systems, whereas no specific central site has been ascribed a primary role in the morphine discriminative cue (*cf.* Joharchi *et al.*, 1993). Moreover, Spyraki *et al.* (1983) found that disruption of dopaminergic transmission by 6-hydroxydopamine-induced lesions of the nucleus accumbens or by haloperidol treatment blocks CPP induced by opioids including morphine. On the other hand, no agreement exists on the involvement of dopaminergic transmission in the mediation of interoceptive properties of opioids. Thus, complete generalization to a morphine cue after amphetamine pretreatment has been reported in some (Shannon and Holtzman, 1979) but not all studies (Joharchi *et al.*, 1993). Similarly, it appears that dopaminergic antagonists do not block the opioid discriminative stimulus (Colpaert *et al.*, 1976; Colpaert, 1977).

The differential involvement of dopaminergic pathways in opioid-induced CPP and drug discrimination of opioid compounds prompt consideration of an interaction between glutamatergic and dopaminergic neurotransmission as a potential basis of the inhibitory effects of memantine on morphine-induced CPP. Several findings indicate that the mesolimbic dopaminergic pathways are the anatomical substrate of drug reinforcement (Di Chiara and Imperato, 1988; Phillips and Le Paine, 1980; also see Koob, 1992; Robinson and Berridge, 1993 for reviews) where dopaminergic and glutamatergic transmission interact mutually (Jedema and Moghaddam, 1994; Kalivas *et al.*, 1989; Krebs *et al.*, 1991; Moghaddam and Bolinao, 1994; Wolf *et al.*, 1994). In the striatum and nucleus accumbens glutamatergic afferents can increase dopamine release through interaction with NMDA receptors (Di Chiara and Imperato, 1988; Krebs *et al.*, 1991). Increased locomotor activity has been observed after intra-accumbens injection of glutamate; this effect is blocked by dopaminergic antagonists (Donzanti and Uretsky, 1983). Because mesolimbic dopaminergic transmission is inevitably involved in the regulation of drug reward (for reviews see Koob, 1992), it is likely that its modulation may affect the reinforcing properties of opioids. It remains intriguing why the noncompetitive NMDA receptor antagonists that have stimulant actions and increase activity of the mesolimbic system, as evidenced by biochemical (Bubser *et al.*, 1992) and electrophysiological data (French, 1994), evoke inhibitory effects on the reinforcing effects of morphine.

In contrast to another uncompetitive NMDA receptor antagonist, dizocilpine that by itself produces clear CPP (Layer *et al.*, 1993; Papp and Moryl, 1994), memantine seemed devoid of these reinforcing effects (fig. 3). This result was unexpected, but it should be noted that dizocilpine exerts this reinforcing effect in a narrow dose range (Layer *et al.*, 1993); and, as already mentioned, the blockade of NMDA receptor channel by memantine is characterized by much faster kinetics (Chen *et al.*, 1992) and stronger voltage dependence (Parsons *et al.*, 1993). In general, it can be argued that lower affinity directly translates into faster channel blocking kinetics and, within a certain range, into a more favorable side-effects profile. However, too low affinity usually results in a loss of selectivity which, in turn, can result in an increase of side effects (see Parsons *et al.*, 1995 for discussion). The dose of memantine effective in the present study (7.5. mg/kg) would be expected to produce serum concentrations of $\sim$1.5 μM (see Danysz *et al.*, 1994 for discussion), and a somewhat (40–50%) lower concentration would be predicted to occur in the cerebrospinal fluid (Danysz *et al.*, 1994). In demented patients treated with memantine, serum concentrations of $\sim$0.4 to 0.5 μM are observed (Kornhuber and Quack, 1995). Based on patch-clamp studies (Parsons *et al.*, 1993) such concentrations should be sufficient to inhibit NMDA receptors in the brain. Hence, the failure to demonstrate CPP after memantine was not caused by insufficient dosing, which is also supported by the finding indicating that neuroprotective activity is observed at doses leading to serum levels of ~1 μM (Misztal *et al.*, 1996; Wenk *et al.*, 1995).

Converging lines of evidence indicate that NMDA receptor antagonists are effective in inhibiting the physical aspects of the expression of morphine dependence (Cappendijk *et al.*, 1993; Popik *et al.*, 1995; Rasmussen *et al.*, 1991; Tanganelli *et al.*, 1991; Trujillo and Akil, 1991). Such effects have also been demonstrated recently for memantine at doses expected to affect primarily, if not solely, NMDA receptors (Popik and Skolnick, 1996). It is worth noting that the inhibitory effects of memantine on the expression of morphine withdrawal was attenuated by glycine administration (Popik and Skolnick, 1996), which suggests the involvement of NMDA receptors. The expression of morphine dependence is typically measured by precipitating morphine withdrawal syndrome with an opioid antagonist. Although contemporary theories of drug addiction do not attribute a major motivational role of physical withdrawal syndrome to the maintenance of opioid dependence (Robinson and Berridge, 1993; Wise and Bozarth, 1987), the abrupt cessation of opioid administration produces a state of extremely unpleasant sensations that may motivate addicts to maintain illicit drug use (Eddy *et al.*, 1965). The difference in physical and psychological aspects of withdrawal syndrome is illustrated by the poor long-term effectiveness of clonidine treatment of abstinence syndrome in humans. Clonidine, an *alpha*-2 adrenergic agonist, decreases the opioid abstinence syndrome in addicted individuals (Gold *et al.*, 1978); however, it principally alleviates the physical but not psychological (motivational) consequences of opioid withdrawal (Charney *et al.*, 1981; Jasinski *et al.*, 1985). It might be hypothesized that the poor overall outcome (*i.e.*, success rate of approximately 40% [Rounsaville *et al.*, 1985]) of clonidine treatment of opioid abuse is caused by the fact that clonidine fails to affect the motivational aspects of opioid withdrawal syndrome. Therefore pharmacological manipulations that would diminish the motivational aspects of opioid withdrawal may be considered as more beneficial treatments of opioid dependence. Such inhibitory effects on motivational aspects of opioid withdrawal syndrome as demonstrated in the CPA procedure have been shown previously for dizocilpine (Higgins *et al.*, 1992) and for memantine in the present experiments (fig. 5). Clinical trials with other uncompetitive NMDA receptor antagonists, dextromethorphan and ibogaine, in opioid addicts were reported to be successful (Koyuncuoglu and Saydam, 1990; Koyuncuoglu, 1995; Sheppard, 1994).

In conclusion, the present study demonstrates that in rats memantine may attenuate the reinforcing aspects of morphine and of morphine withdrawal syndrome, being inactive by itself in the CPP or CPA paradigms. It remains to be established if the diminution of the physical (Popik and Skolnick, 1996) as well as motivational (present study) signs of expression of opioid withdrawal produced by memantine also can be seen in humans.

Acknowledgments

The authors would like to thank Dr. Parsons for linguistic corrections.

References

Adams, M. L., Morris, D. L., Brase, D. A. and Dewey, W. L.: Stereoselective effect of morphine on antinociception and endogenous opioid peptide levels in plasma but not cerebrospinal fluid of dogs. Life Sci. **48:** 917–924, 1991.

Ball, J. C. and Ross, A.: Follow-up study of 105 patients who left treatment. *In* The Effectiveness of Methadone Maintenance Treatment. Patients, Programs, Services, and Outcome, ed. by J. C. Ball and A. Ross, pp. 176–187, Springer-Verlag, New York, 1991.

Barnes, C. A., Danysz, W. and Parsons, C. G.: Effects of the uncompetitive NMDA receptor antagonist memantine on hippocampal long-term potentiation, short-term exploratory modulation and spatial memory in awake, freely moving rats. Eur. J. Neurosci. **8:** 565–571, 1996.

Ben-Eliyahu, S., Marek, P., Vaccarino, A. L., Mogil, J. S., Sternberg, W. F. and Liebeskind, J. C.: The NMDA receptor antagonist MK-801 prevents long-lasting non-associative morphine tolerance in the rat. Brain Res. **575:** 304–308, 1992.

Bespalov, A., Dumpis, M., Piotrovsky, L. and Zvartau, E.: Excitatory amino acid receptor antagonist kynurenic acid attenuates rewarding potential of morphine. Eur. J. Pharmacol. **264:** 233–239, 1994.

Bespalov, A., Zvartau, E. E. and Beardsley, P. M.: Kynurenic acid attenuates rewarding potential but fails to alter discriminative stimulus properties of opiates in rodents. Fed. Eur. Pharmacol. Soc. (Milan) **31:** 113, 1995.

Bhargava, H. N. and Matwyshyn, G. A.: Dizocilpine (MK-801) blocks tolerance to the analgesic but not to the hyperthermic effect of morphine in the rat. Pharmacology **47:** 344–350, 1993.

Bindra, D.: How adaptive behavior is produced: A perceptual-motivation alternative to response reinforcement. Behav. Brain Sci. **1:** 41–91, 1978.

Bormann, J.: Memantine is a potent blocker of N-methyl-D-aspartate (NMDA) receptor channels. Eur. J. Pharmacol. **166:** 591–592, 1989.

Brent, P. J. and Chahl, L. A.: Enhancement of the opiate withdrawal response by antipsychotic drugs in guinea-pigs is not mediated by sigma binding sites. Eur. Neuropsychopharmacol. **3:** 23–32, 1993.

Bubser, M., Keseberg, U., Notz, P. K. and Schmidt, W. J.: Differential behavioural and neurochemical effects of competitive and non-competitive NMDA receptor antagonists in rats. Eur. J. Pharmacol. **229:** 75–82, 1992.

Cappendijk, S. L., de Vries, R. and Dzoljic, M. R.: Excitatory amino acid receptor antagonists and naloxone-precipitated withdrawal syndrome in morphine-dependent mice. Eur. Neuropsychopharmacol. **3:** 111–116, 1993.

Caramanos, Z. and Shapiro, M. L.: Spatial memory and N-methyl-D-aspartate receptor antagonists APV and MK-801-memory impairments depend on familiarity with the environment, drug dose, and training duration. Behav. Neurosci. **108:** 30–43, 1994.

Carr, G. F., Fibiger, H. C. and Phillips, A. G.: Conditioned place preference as a measure of drug reward. *In* The Neuropharmacological Basis of Reward, ed. by J. Liebman and S. J. Cooper, pp. 264–319, Clarendon Press, Oxford, 1989.

Charney, D. S., Sternberg, D. E., Kleber, H. D., Heninger, G. R. and Redmond, D. E. Jr.: The clinical use of clonidine in abrupt withdrawal from methadone. Effects on blood pressure and specific signs and symptoms. Arch. Gen. Psychiatry **38:** 1273–1277, 1981.

Chen, H. S. V., Pellegrini, J. W., Aggarwal, S. K., Lei, S. Z., Warach, S., Jensen, F. E. and Lipton, S. A.: Open-channel block of N-methyl-D-aspartate (NMDA) responses by memantine: Therapeutic advantage against NMDA receptor-mediated neurotoxicity. J. Neurosci. **12:** 4427–4436, 1992.

Colpaert, F. C.: Narcotic cue and narcotic state. Life Sci. **20:** 1097–1108, 1977.

Colpaert, F. C., Lal, H., Niemegeers, C. J. and Janssen, P. A.: Investigations on drug produced and subjectively experienced discriminative stimuli. I. The fentanyl cue, a tool to investigate subjectively. Life Sci. **16:** 705–715, 1975.

Colpaert, F. C., Niemegeers, C. J. E. and Janssen, P. A. J.: Fentanyl and apomorphine: Assymetrical generalisation of discriminative stimulus properties. Neuropharmacology **15:** 541–545, 1976.

Danysz, W., Gossel, M., Zajaczkowski, W., Dill, D. and Quack, G.: Are NMDA antagonistic properties relevant for antiparkinsonian-like activity in rats? Case of amantadine and memantine. J. Neural Transm. Park. Dis. Dement. Sect. **7:** 155–166, 1994.

Danysz, W., Parsons, C. G., Bresink, I. and Quack, G.: Glutamate in CNS disorders: A revived target for drug development. Drug News Perspect. **8:** 261–277, 1995a.

Danysz, W., Zajaczkowski, W. and Parsons, C. G.: Modulation of learning processes by ionotropic glutamate receptor ligands. Behav. Pharmacol. **6:** 455–474, 1995b.

Di Chiara, G. and Imperato, A.: Drugs abused by humans preferentially increase synaptic dopamine concentratin in the mesolimbic system of freely moving rats. Proc. Natl. Acad. Sci. U.S.A. **85:** 5274–5278, 1988.

Ditzler, K.: Efficacy and tolerability of memantine in patients with dementia syndrome. A double blind, placebo controlled trial. Arzneimittelforschung **41:** 773–780, 1991.

Donzanti, B. A. and Uretsky, N. J.: Effects of excitatory amino acids on locomotor activity after bilateral microinjection into the rat nucleus accumbens: Possible dependence on. Neuropharmacology **22:** 971–981, 1983.

Eddy, N. B., Halbach, H., Isbell, H. and Seevers, M. H.: Drug dependence: Its significance and characteristics. Bull. W.H.O. **32:** 721–733, 1965.

Elliott, K., Minami, N., Kolesnikov, Y. A., Pasternak, G. W. and Inturrisi, C. E.: The NMDA receptor antagonists, LY74614 and MK801, and the nitric oxide synthase inhibitor, ng-nitro-l-arginine, attenuate analgesic tolerance to the μ-opioid morphine but not to κ-opioids. Pain **56:** 69–75, 1994.

File, S. E. and Fernandes, C.: Dizocilpine prevents the development of tolerance to the sedative effects of diazepam in rats. Pharmacol. Biochem. Behav. **47:** 823–826, 1994.

French, E. D.: Phencyclidine and the midbrain dopamine system:- Electrophysiology and behavior. Neurotoxicol. Teratol. 16: 355–362, 1994.

Gianutsos, G. and Lal, H.: Effect of loperamide, haloperidol and methadone in rats trained to discriminate morphine from saline. Psychopharmacologia 41: 267–270, 1975.

Gianutsos, G. and Lal, H.: Selective interaction of drugs with a discriminable stimulus associated with narcotic action. Life Sci. 19: 91–98, 1976.

Gold, M. S., Redmond, E. D. and Kleber, H. D.: Clonidine blocks acute opiate withdrawal symptoms. Lancet 2: 599–601, 1978.

Goudie, A. J.: Animal models of drug abuse and dependence. *In* Behavioural Models in Psychopharmacology: Theoretical, Industrial and Clinical Perspectives, ed. by P. Willner, pp. 453–484, Cambridge University Press, Cambridge, 1991.

Görtelmeyer, R. and Erbler, H.: Memantine in the treatment of mild to moderate dementia syndrome: A double-blind placebo-controlled study. Arzneimittelforschung 42: 904–913, 1992.

Henriksson, B. G. and Jarbe, T. U. C.: Delta 9-Tetracannabinol used as discriminative stimulus for rats in position learning in a T-shaped water maze. Psychonom. Sci. 27: 25–26, 1972.

Higgins, G. A., Nguyen, P., Joharchi, N. and Sellers, E. M.: Effects of 5-HT$_3$ receptor antagonists on behavioural measures of naloxone-precipitated opioid withdrawal. Psychopharmacology 105: 322–328, 1991.

Higgins, G. A., Nguyen, P. and Sellers, E. M.: The NMDA antagonist dizocilpine (MK801) attenuates motivational as well as somatic aspects of naloxone precipitated opioid withdrawal. Life Sci. 50: PL167–PL172, 1992.

Jaffe, J. H.: Pharmacological agents in treatment of drug dependence. *In* Psychopharmacology. The Third Generation of Progress, ed. by H. Y. Meltzer, pp. 1605–1616, Raven Press, New York, 1987.

Jarbe, T. U. C.: Drug discrimination learning. Cue properties of drugs. *In* Experimental Psychopharmacology, pp. 433–479, The Humana Press, Clifton, NJ, 1987.

Jasinski, D. R., Johnson, R. E. and Kocher, T. R.: Clonidine in morphine withdrawal. Differential effects on signs and symptoms. Arch. Gen. Psychiatry 42: 1063–1066, 1985.

Jedema, H. P. and Moghaddam, B.: Glutamatergic control of dopamine release during stress in the rat prefrontal cortex. J. Neurochem. 63: 785–788, 1994.

Joharchi, N., Sellers, E. M. and Higgins, G. A.: Effect of 5-HT$_3$ receptor antagonists on the discriminative stimulus properties of morphine in rats. Psychopharmacology 112: 111–115, 1993.

Kalivas, P. W., Duffy, P. and Barrow, J.: Regulation of the mesocorticolimbic dopamine system by glutamic acid receptor subtypes. J. Pharmacol. Exp. Ther. 251: 378–387, 1989.

Khanna, J. M., Kalant, H., Shah, G. and Chau, A.: Effect of D-cycloserine on rapid tolerance to ethanol. Pharmacol. Biochem. Behav. 45: 983–986, 1993.

Kolesnikov, Y. A., Ferkany, J. and Pasternak, G. W.: Blockade of μ and κ_1 opioid analgesic tolerance by NCP17742, a novel NMDA antagonist. Life Sci. 53: 1489–1494, 1993.

Kolesnikov, Y. A., Maccecchini, M. L. and Pasternak, G. W.: 1-aminocyclopropanecarboxylic acid (ACPC) prevents μ and δ opioid tolerance. Life Sci. 55: 1393–1398, 1994.

Koob, G. F.: Neurobiological mechanisms in cocaine and opiate dependence. *In* Addictive States, ed. by C. P. O'Brien and J. H. Jaffe, pp. 79–92, Raven Press Ltd, New York, 1992.

Kornhuber, J., Bormann, J., Retz, W., Hubers, M. and Riederer, P.: Memantine displaces [^{3}H]MK-801 at therapeutic concentrations in postmortem human frontal cortex. Eur. J. Pharmacol. 166: 589–590, 1989.

Kornhuber, J. and Quack, G.: Cerebrospinal fluid and serum concentrations of the N-methyl-D-aspartate (NMDA) receptor antagonist memantine in man. Neurosci. Lett. 195: 137–139, 1995.

Koyuncuoglu, H.: The combination of tizanidine markedly improves the treatment with dextromethorphan of heroin addicted outpatients. Int. J. Clin. Pharmacol. Ther. Toxicol. 33: 13–19, 1995.

Koyuncuoglu, H. and Saydam, B.: The treatment of heroin addicts with dextromethorphan. A double-blind comparison of dextromethorphan with chloropromazine. Int. J. Clin. Pharmacol. Ther. Toxicol. 28: 147–152, 1990.

Krebs, M. O., Desce, J. M., Kemel, M. L., Gauchy, C., Godeheu, G., Cheramy, A. and Glowinski, J.: Glutamatergic control of dopamine release in the rat stroatum: Evidence for presynaptic N-methyl-D-aspartate receptors on dopaminergic nerve terminals. J. Neurochem. 56: 81–85, 1991.

Kutsuwada, T., Kashiwabuchi, N., Mori, H., Sakimura, K., Kushiya, E., Araki, K., Meguro, H., Masaki, H., Kumanishi, T., Arakawa, M. and Mishina, M.: Molecular diversity of the NMDA receptor channel. Nature 358: 36–41, 1992.

Layer, R. T., Kaddis, F. G. and Wallace, L. J.: The NMDA receptor antagonist MK-801 elicits conditioned place preference in rats. Pharmacol. Biochem. Behav. 44: 245–247, 1993.

Litchfield, J. T. and Wilcoxon, F.: A simplified method of a evaluating dose-effects experiments. J. Pharmacol. Exp. Ther. 96: 99–133, 1949.

Lotsof, H. S.: Ibogaine in the treatment of chemical depencency disorders: Clinical perspectives. Bull. Multidisciplinary Association for Psychedelic Studies 5: 16–27, 1995.

Luby, E. D., Cohen, R. C., Rosenbaum, B., Gottlieb, J. S. and Kelly, R.: Study of a new schizophrenomimetic drug: Sernyl. Arch. Neurol. Psychiatry 81: 363–369, 1959.

Martin, W. R. and Jasinski, D. R.: Physiological parameters of morphine dependence in man–tolerance, early abstinence, protracted abstinence. J. Psychiatr. Res. 7: 9–17, 1969.

Misztal, M. and Danysz, W.: Comparison of glutamate antagonists in continuous multiple-trial and single-trial dark avoidance. Behav. Pharmacol. 6: 550–561, 1995.

Misztal, M., Frankiewicz, T., Parsons, C. G. and Danysz, W.: Learning deficits induced by chronic intraventricular infusion of quinolinic acid: Protection by MK-801 and memantine. Eur. J. Pharmacol. 296: 1–8, 1996.

Moghaddam, B. and Bolinao, M. L.: Glutamatergic antagonists attenuate ability of dopamine uptake blockers to increase extracellular levels of dopamine: Implications for tonic. Synapse 18: 337–342, 1994.

Mucha, R. F. and Herz, A.: Motivational properties of kappa and μ opioid receptor agonists studied with place and taste preference conditioning. Psychopharmacology 86: 274–280, 1985.

Mucha, R. F., Van der Kooy, D., O'Shaughnessy, M. and Bucenieks, P.: Drug reinforcement studied by the use of place conditioning in the rat. Brain Res. 243: 91–105, 1982.

Pani, L., Kuzmin, A., Martellotta, M. C., Gessa, G. L. and Fratta, W.: The calcium antagonist PN 200–110 inhibits the reinforcing properties of cocaine. Brain Res. Bull. 26: 445–447, 1991.

Papp, M. and Moryl, E.: Rewarding properties of noncompetitive and competitive NMDA antagonists as measured by place preference conditioning in rats. Pol. J. Pharmacol. Pharm. 46: 79–81, 1994.

Parsons, C. G., Gruner, R., Rozental, J., Millar, J. and Lodge, D.: Patch clamp studies on the kinetics and selectivity of N-methyl-D-aspartate receptor antagonism by memantine (1-amino-3,5-dimethyladamantan). Neuropharmacology 32: 1337–1350, 1993.

Parsons, C. G., Quack, G., Bresink, I., Baran, L., Przegalinski, E., Kostowski, W., Krzascik, P., Hartmann, S. and Danysz, W.: Comparison of the potency, kinetics and voltage-dependency of a series of uncompetitive NMDA receptor antagonists *in vitro* with anticonvulsive and motor impairment activity *in vivo*. Neuropharmacology 34: 1239–1258, 1995.

Phillips, A. G. and Le Paine, F. G.: Reinforcing effects of morphine microinjection into the ventral tegmental area. Pharmacol. Biochem. Behav. 12: 965–968, 1980.

Pizzi, W. J. and Cook, D. F.: Conditioned taste aversion is a confound in behavioral studies that report a reduction in the reinforcing effects of drugs. Pharmacol. Biochem. Behav. 53: 243–247, 1996.

Popik, P., Layer, R. T., Fossom, L., Benveniste, M., Getter-Douglas, B., Witkin, J. M. and Skolnick, P.: NMDA antagonist properties of the putative antiaddicitive drug, ibogaine. J. Pharmacol. Exp. Ther. 275: 753–760, 1995.

Popik, P., Mamczarz, J. and Vetulani, J.: The effect of electroconvulsive shock and nifedipine on spatial learning and memory in rats. Biol. Psychiatry 35: 864–869, 1994a.

Popik, P., Nalepa, I., Mamczarz, J. and Vetulani, J.: Retrieval associated cholinergic activity and its inhibition by memory updating. Life Sci. 54: 1251–1257, 1994b.

Popik, P. and Skolnick, P.: The NMDA antagonist memantine blocks the expression and maintenance of morphine dependence. Pharmacol. Biochem. Behav. 53: 791–798, 1996.

Pudiak, C. M. and Bozarth, M. A.: L-NAME and MK-801 attenuate sensitization to the locomotor-stimulating effect of cocaine. Life Sci. 53: 1517–1524, 1993.

Pulvirenti, L. and Koob, G. F.: Dopamine receptor agonists, partial agonists and psychostimulant addiction. Trends Pharmacol. Sci. 15: 374–379, 1994.

Rasmussen, K., Fuller, R. W., Stockton, M. E., Perry, K. W., Swinford, R. M. and Ornstein, P. L.: NMDA receptor antagonists supress behaviors but not norepinephrine turnover or locus coeruleus unit activity induced by opiate withdrawal. Eur. J. Pharmacol. 197: 9–16, 1991.

Rice, K. C., Jacobson, A. E., Burke, T. R. Jr., Bajwa, B. S., Streaty, R. A. and Klee, W. A.: Irreversible ligands with high selectivity toward delta and μ opiate receptors. Science 220: 314–316, 1983.

Robinson, T. E. and Berridge, K. C.: The neural basis of drug craving: an incentive-sensitization theory of addiction. Brain Res. Rev. 18: 247–291, 1993.

Rogawski, M. A.: Therapeutic potential of excitatory amino acid antagonists: Channel blockers and 2,3-benzodiazepines. Trends Pharmacol. Sci. 14: 325–331, 1993.

Rounsaville, B. J., Kosten, T. R. and Kleber, H. D.: Success and failure at outpatient opioid detoxification: evaluating the process of clonidine- and methadone-assisted withdrawal. J. Nerv. Ment. Dis. 173: 103–110, 1985.

Shannon, H. E. and Holtzman, S. G.: Morphine training dose: a determinant of stimulus generalization to narcotic antagonists in the rat. Psychopharmacology 61: 239–244, 1979.

Shapiro, M. L. and Caramanos, Z.: NMDA antagonist MK-801 impairs acquisition but not performance of spatial working and reference memory. Psychobiology 18: 231–243, 1990.

Sheppard, S. G.: A preliminary investigation of ibogaine: Case reports and recommendations for further study. J. Subst. Abuse Treat. 11: 379, 1994.

Shoaib, M. and Stolerman, I. P.: MK-801 attenuates behavioural adaptation to chronic nicotine administration in rats. Br. J. Pharmacol. 105: 514–515, 1992.

Spyraki, C., Fibiger, H. C. and Phillips, A. G.: Attenuation of heroin reward in rats by disruption of the mesolimbic dopamine system. Psychopharmacology 79: 278–283, 1983.

TANGANELLI, S., ANTONELLI, T., MORARI, M., BIANCHI, C. AND BEANI, L.: Glutamate antagonists prevent morphine withdrawal in mice and guinea pigs. Neurosci. Lett. **122:** 270–272, 1991.

TISEO, P. J., CHENG, J., PASTERNAK, G. W. AND INTURRISI, C. E.: Modulation of morphine tolerance by the competitive N-methyl-D-aspartate receptor antagonist LY274614: assessment of opioid receptor. J. Pharmacol. Exp. Ther. **268:** 195–201, 1994.

TISEO, P. J. AND INTURRISI, C. E.: Attenuation and reversal of morphine tolerance by the competitive N-methyl-D-aspartate receptor antagonist, LY274614. J. Pharmacol. Exp. Ther. **264:** 1090–1096, 1993.

TROUPIN, A. S., NENDIUS, J. R., CHENG, F. AND RISINGER, M. W.: MK-801. *In* New Anticonvulsant Drugs, ed. by B. S. Meldrum and R. J. Porter, pp. 191–201, John Libbey, London, 1986.

TRUJILLO, K. A. AND AKIL, H.: Inhibition of morphine tolerance and dependence by the NMDA receptor antagonist MK-801. Science **251:** 85–87, 1991.

TZSCHENTKE, T. M. AND SCHMIDT, W. J.: N-methyl-D-aspartic acid-receptor antagonists block morphine-induced conditioned place preference in rats. Neurosci. Lett. **193:** 37–40, 1995.

VAN DER KOOY, D., MUCHA, R. F., O'SHAUGHNESSY, M. AND BUCENIEKS, P.: Reinforcing effects of brain microinjections of morphine revealed by conditioned place preference. Brain Res. **243:** 107–117, 1982.

WENK, G. L., DANYSZ, W. AND MOBLEY, S. L.: MK-801, memantine and amantadine show neuroprotective activity in the nucleus basalis magnocellularis. Eur. J. Pharmacol. Environ. Toxicol. Pharmacol. **293:** 267–270, 1995.

WESSINGER, W. D.: Tolerance to and dependence on MK-801 (dizocilpine) in rats. Pharmacol. Biochem. Behav. **49:** 1049–1056, 1994.

WHITE, N. M. AND CARR, G. D.: The conditioned place preference is affected by two independent reinforcement processes. Pharmacol. Biochem. Behav. **23:** 37–42, 1985.

WIKLER, A., MARTIN, W. R., PESCOR, F. T. AND EADES, C. G.: Factors regulating oral consumption of an opioid (Etonitazene) by morphine-addicted rats. Psychopharmacologia **5:** 55–76, 1963.

WISE, R. A. AND BOZARTH, M. A.: A psychomotor stimulant theory of addiction. Psychol. Rev. **94:** 469–492, 1987.

WOLF, M. E. AND KHANSA, M. R.: Repeated administration of MK-801 produces sensitization to its own locomotor stimulant effects but blocks sensitization to amphetamine. Brain Res. **562:** 164–168, 1991.

WOLF, M. E., WHITE, F. J. AND HU, X. T.: MK-801 prevents alterations in the mesoaccumbens dopamine system associated with behavioral sensitization to amphetamine. J. Neurosci. **14:** 1735–1745, 1994.

WOLFER, D. P. AND LIPP, H. P.: A new computer program for detailed off-line analysis of swimming navigation in the Morris water maze. J. Neurosci. Methods **41:** 65–74, 1992.

YOUNG, A. M., MASAKI, M. A. AND GEULA, C.: Discriminative stimulus effects of morphine: effects of training dose on agonist and antagonist effects of μ opioids. J. Pharmacol. Exp. Ther. **261:** 246–257, 1992.

ZAJACZKOWSKI, W., QUACK, G. AND DANYSZ, W.: Infusion of (+)-MK-801 and memantine: Contrasting effects on radial maze learning in rats with entorhinal cortex lesion. Eur. J. Pharmacol. **296:** 239–246, 1996.

Send reprint requests to: Dr. Piotr Popik, Institute of Pharmacology, Polish Academy of Sciences, Smętna 12, 31–343 Kraków, Poland. E-mail: nfpopik@cyf-kr.edu.pl